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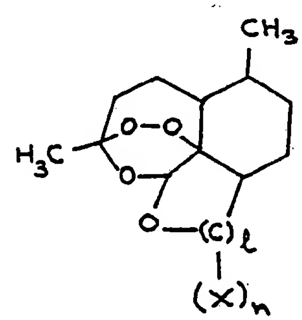
(54) Title: CYCLIC PEROXYACETAL COMPOUNDS

(57) Abstract

Compounds of general formula (I), pharmaceutically acceptable salts thereof or stereoisomeric forms thereof, wherein l = 1, 2 or 3, n is an integer from 1-6, where X is independently selected from H, =O, $=CH_2$, aryl, COR, OR, COOR or X = $-(CR_1R_2)_r-R_3$ where r is an integer from 1-10 and where r > 1, optionally at least 1 carbon atom can be replaced by O, S or N; R_1, R_2 and R_3 are independently selected from H; alkyl, alkenyl, alkynyl, aryl, each optionally substituted by one or more substituents selected from alkyl, alkenyl, alkynyl, aryl, halogen, OR, CF_3, NO_2, COOR, NRR′, SR, COR, CONRR′, SO_3R, SO_2NRR', SR, SOR, and SO_2R, where R and R′ are independently selected from H, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted aryl or optionally substituted arylalkyl wherein the optional substituents are as defined above. These compounds are structurally similar to the naturally occurring biologically active compound qinghaosu (Artemisinin) and some compounds have activities superior to that of qinghaosu.



(I)

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AT	Austria
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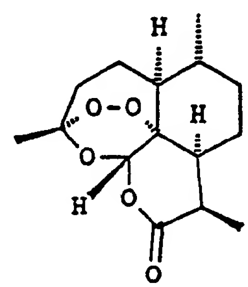
CYCLIC PEROXYACETAL COMPOUNDS

Technical Field

This invention relates to novel cyclic peroxyacetal compounds and in particular relates to compounds related to qinghaosu (Artemisinin) which is a naturally occurring biologically active compound.

Background of the Invention

The present invention provides novel compounds which are structurally similar to the naturally occurring biologically active compound qinghaosu (Artemisinin) which has the following formula:



Qinghaosu is a potent anti-malarial which has been successfully used to treat patients suffering from malaria. The re-emergence of strains of malaria resistant to conventional (chloroquine) therapy is posing a world-wide problem and indeed, there is no universally acceptable cure at the present time. Qinghaosu occurs to the extent of about 0.1% (dry weight) in an annual shrub, qinghao or *Artemisia annua*, which grows in most provinces of China. Unfortunately, the world demand for qinghaosu far exceeds the supply, and there is considerable pressure to develop bioactive analogues and derivatives or to develop alternative sources for the compound.

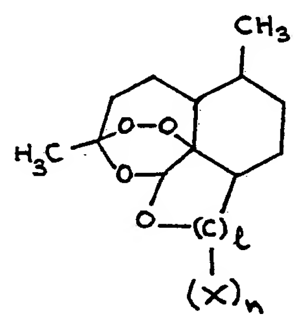
Some of the novel compounds have activities superior to that of qinghaosu and furthermore these compounds will

be suitable for preparing conjugate drugs for the treatment of malaria and other parasitic and viral diseases.

The novel compounds can also be used as building blocks because of their reactive side chain and other active drugs can be linked via these side chains to form conjugate drugs.

Disclosure of the Invention

In one aspect, the present invention provides compounds of general formula (I), pharmaceutically acceptable salts thereof or stereoisomeric forms thereof



(I)

wherein

l = 1, 2 or 3

n is an integer from 1-6

where X is independently selected from

H, =O, $=CH_2$, aryl, COR, OR, COOR or

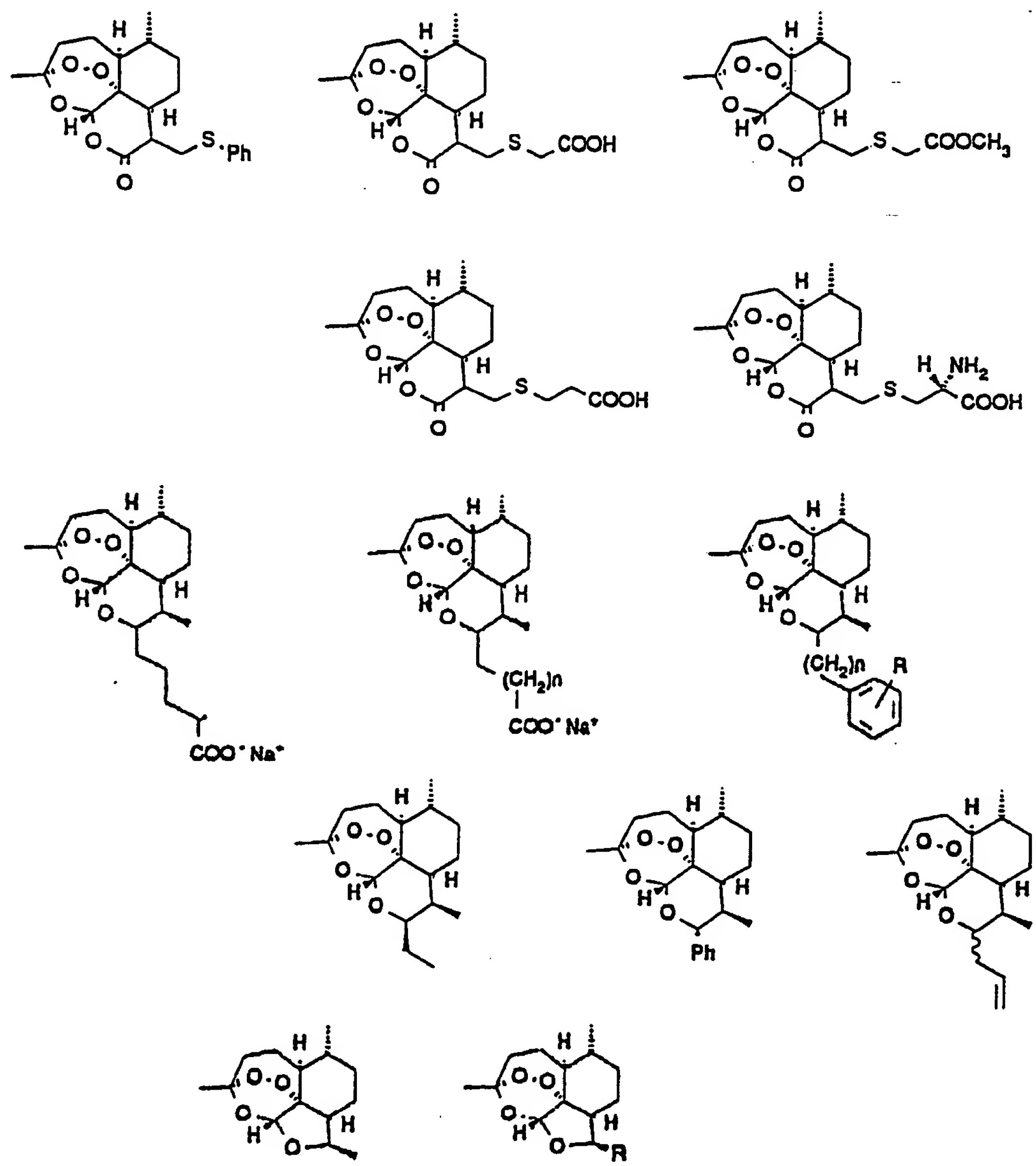
X = $-(CR_1R_2)_r-R_3$ where r is an integer from 1-10 and where r > 1, optionally at least 1 carbon atom can be replaced by O, S or N;

R_1, R_2 and R_3 are independently selected from

H; alkyl, alkenyl, alkynyl, aryl, each optionally substituted by one or more substituents selected from alkyl, alkenyl, alkynyl, aryl, halogen, OR, CF_3, NO_2, COOR, NRR', SR, COR, CONRR', SO_3R, SO_2NRR', SR, SOR, and

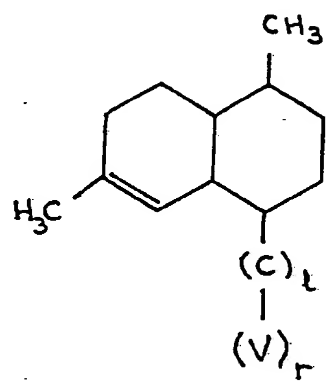
SO_2R, where R and R' are independently selected from H, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted aryl or optionally substituted arylalkyl wherein the optional substituents are as defined above.

Preferred compounds of formula (I) have the following structural formulae:



In another aspect the present invention provides a process for preparing compounds of formula (I), pharmaceutically acceptable salts thereof or stereoisomeric forms thereof comprising:

(A) carrying out at least one addition reaction on a compound of formula (II) having a -C=O functionality



(II)

to provide another compound of formula (II) with an alcoholic functionality at the $(C)_l$-$(V)_r$ group and then followed by oxygenation to give a compound of formula (I); where V is as defined for X; r and l are as defined above;

(B) directly oxygenating a compound of formula (II) where there is a carboxylic functionality at the $(C)_l$-$(V)_r$ reactive chain and also a further functional group to facilitate subsequent addition reaction, to provide compounds of formula (I) and then carrying out a suitable addition reaction to provide the required side chain; or

(C) for compounds of formula (I) where l=1; carrying out one or more of the following steps on a compound of general formula (II):

(i) oxidative degradation and

(ii) reduction

followed by oxygenation to give compounds of formula (I).

Best Mode of Carrying out the Invention

The oxygenation step is basically, the process described in PCT/AU90/00456 (WO 91/04970) and that disclosure is incorporated herein by reference. The oxygenation is preferably carried out as a "one-pot" reaction and involves oxygenation of a compound of formula (II) to provide a hydroperoxide derivative and without isolation further oxygenation in the presence of one or more oxygenating metal catalysts to give a compound of formula (I). The oxygenation of the hydroperoxy compound in the presence of one or more catalysts provides an oxygenation-cleavage-cyclization reaction to give the cyclic peroxyacetal compounds of formula (I).

The oxygenation-cleavage-cyclization reaction is typically carried out by treating with one or more transition metal catalysts such as $Cu(OSO_2CF_3)_2$, Cu(II) propionate, copper(II) 2-ethylhexanoate, other copper(II) carboxylic salts, and various iron(III) salts such as $Fe(phenanthroline)_3(PF_6)_3$. Other catalysts that may be used are cobalt(II) and cobalt(III) salts. Preferably this reaction is carried out in a solvent such as a mixture of acetonitrile and dichloromethane and by treating with one of the above mentioned catalysts, or with a combination of the copper and iron catalysts. Other suitable solvents include hexane, ethyl acetate and the like.

Preferred solvents for the Grignard addition

reactions are diethyl ether, or THF but any other solvent such as benzene or other ether solvent would be suitable.

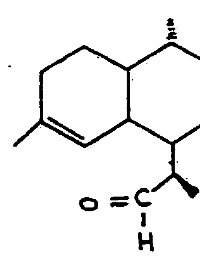
The reaction is typically initially carried out at the temperature of 0^{o}-$5^{o}C$ and continued at room temperature.

Other addition reactions are typically carried out in $CHCl_3$ and CH_2Cl_2 with an amine base. MeOH and aqueous MeOH may also be used and bases such as Na_2CO_3, K_2CO_3 or $NaHCO_3$ can also be used. The reactions are preferably carried out at room temperature.

Oxidative degradation is preferably carried out using cupric acetate, 2,2'-bipyridyl and DABCO in DMF under atmospheric oxygen at between about 70^{o}-$75^{o}C$ for about 12 hours.

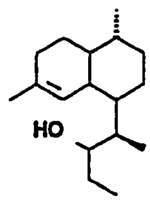
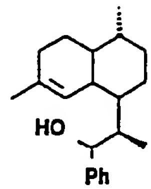
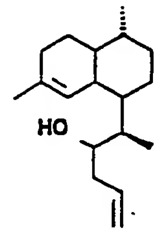
The reduction is preferably carried out in methanol or other alcoholic solvent with $NaBH_4$ at about $0^{o}C$. Other reducing agents with appropriate solvents may also be used.

Preferably the starting compound for process A is the aldehyde 2 having the following structure

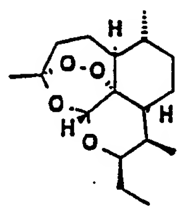
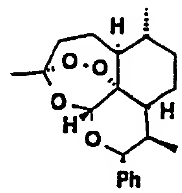
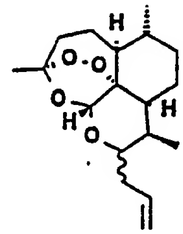


2

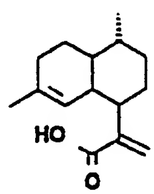
This is preferably treated with Grignard reagents or other organometallic reagents such as those derived from lithium, copper or zinc to provide compounds of the following formulae:

which are then oxygenated to provide the corresponding compounds of formula (I):

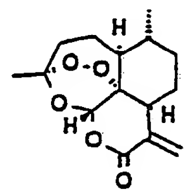
The aldehyde is preferably prepared from qinghao acid which has the following formula:



Qinghao acid occurs to the extent of 1-3% (dry weight) in *Artemisia annua*, which is much greater than the natural occurrence of qinghaosu and is easily extracted from the plant.

The starting material for the process B is preferably

qinghao acid which is oxygenated to provide dehydro-qinghaosu of the following formula



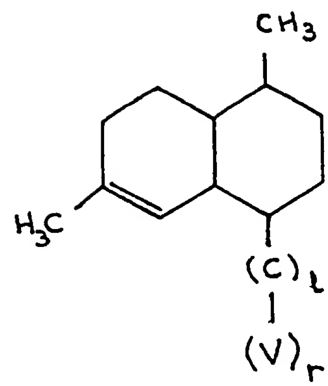
on which is carried out various addition reactions to provide compounds of formula (I).

The starting material for process C is also preferably the aldehyde having the structural formula 2. This aldehyde is typically prepared from qinghao acid by carrying out the following steps.

1. methylation to provide the unsaturated methyl ester;
2. reduction to provide the saturated methyl ester;
3. reduction to provide the alcohol;
4. oxidation to provide the aldehyde.

The skilled addressee would understand that the process of the invention may result in one or more stereogenic (chiral) centres being formed resulting in stereoisomers. Thus it is to be understood that the present invention includes within its scope the preparation of stereoisomers and also encompasses any isomers per se or mixture thereof.

Most of the starting compounds of formula (II) are also novel and form part of the present invention. Accordingly, in yet another aspect, the present invention provides compounds of general formula (II)



(II)

wherein V is as defined for X; r and l are as hereinbefore defined; provided that the $(C)_l$-$(V)_r$ group is not $CH(CH_3)CH_2OH$ and $CH(CH_3)C(=O)H$.

The present invention also provides pharmaceutical compositions comprising a compound of formula (I), a pharmaceutically acceptable salt thereof or stereoisomeric forms thereof in a pharmaceutically acceptable carrier and/or diluent.

Pharmaceutical compositions containing a compound of formula (I) as the active ingredient in admixture with a pharmaceutically acceptable carrier or diluent can be prepared according to conventional pharmaceutical formulating techniques. The carrier may be of any form depending on the form of preparation desired for administration, eg intravenous, oral or parenteral.

In yet another aspect, the present invention provides a method of treatment or prophylaxis of parasitic or viral diseases in a mammal comprising administering to the mammal a compound of formula (I), a pharmaceutically acceptable salt thereof or a stereoisomeric form thereof.

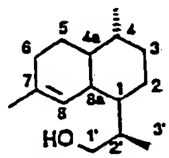
Specific embodiments of the present invention are illustrated by the following preparative examples. It will be understood, however, that the invention is not confined to the specific limitations set forth in the individual examples.

PREPARATION OF STARTING COMPOUNDS

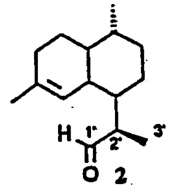
Preparation of Derivatives of Qinghao Acid

<u>Example 1</u>

a) Dihydroqinghao alcohol (1) and dihydroqinghao aldehyde (2)



1



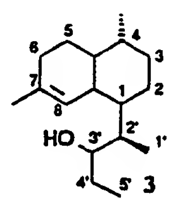
2

Qinghao acid (307 mg; 1.31 mmol) was firstly converted into its methyl ester by heating it with dimethyl sulfate (198 mg; 1.57 mmol; 0.15 ml) and potassium carbonate (199 mg; 1.44 mmol) in acetone (15 ml) under gentle reflux for 2 h. The crude product was then dissolved in methanol (7 ml) and treated with sodium borohydride (72 mg; 1.90 mmol) in the presence of nickel boride, which was preformed from $NiCl_2.6H_2O$ (30 mg; 0.13 mmol), at -30^0 to give the saturated ester after acidic workup. This was then dissolved in diethyl ether (7 ml) and treated with lithium aluminium hydride (1 M solution in THF), with cooling in ice, until complete conversion to the saturated alcohol was observed by t.l.c. The reaction mixture was heated under gentle reflux for 40 min. before being cooled and quenched with ethyl acetate (5 ml) and water (2 ml). Sodium sulfate was added to the stirred mixture until the excess water had been absorbed and it was then removed by filtration, and washed with more ether. The filtrate was evaporated to dryness to give dihydroqinghao alcohol (1). The pure alcohol could be isolated by flash chromatography (ether/light petroleum, 40:60) in greater than 90% yield, ^1H NMR spectrum (200 MHz, $CDCl_3$) δ 0.864 (3H, d, $J_{4\text{-Me},4}$ = 6.4 Hz, 4-CH_3), 0.997 (3H, d, $J_{3',2'}$ = 6.8 Hz, H3'), 1.630 (3H, m, 7-CH_3), 2.475 (1H, br s, $W_{h/2}$ = 11.5 Hz, OH), 3.522 (1H, dd, J_{gem}=10.6, $J_{1',2'}$ = 6.2 Hz, H1'), 3.740 (1H, dd, J_{gem} = 10.6, $J_{1',2'}$ =3.3 Hz, H1'), 5.218 (1H, m, H8). However, in

general, the residue after evaporation of the filtrate, without purification, was immediately submitted to Swern oxidation conditions. Thus, DMSO (0.37 ml; 5.2 mmol) in dichloromethane (2 ml) was added dropwise to a solution of oxalyl chloride (0.23 ml; 2.6 mmol) in dichloromethane (8 ml) at between -50° and -60°. After 5 min. the crude alcohol (288 mg) in dichloromethane (4 ml) was added dropwise at the same temperature and after 15 min. triethylamine (1.09 ml; 7.8 mmol) was added. Stirring was continued at -60° for 5 min. and then the whole was allowed to warm to room temperature over 20 min. Aqueous workup afforded the aldehyde (2) which was submitted to flash chromatography (ether/light petroleum, 5:95) to give a colourless oil [228 mg; 79% from qinghao acid]. The aldehyde was found to be a 6.2:1 mixture of diastereomers and as such was used to prepare the other qinghao acid derivatives. ^{1}H NMR spectrum (200 MHz, $CDCl_3$) δ 0.877 (3H, d, $J_{4\text{-Me},4}$ = 6.3 Hz, 4-CH_3), 1.067 (3H, d, $J_{3',2'}$ = 7.0 Hz, H3'), 1.638 (3H, m, 7-CH_3), 5.132 (1H, m, H8), 9.588 (1H, d, $J_{1',2'}$ = 4.2 Hz, H1').

<u>Example 2</u>

b) Ethyl alcohol (3)



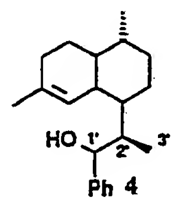
3

The aldehyde (50.6 mg; 0.23 mmol) was dissolved in diethyl ether (3 ml) and treated with ethyl magnesium bromide in ether with cooling in an ice bath. The whole was then stirred at room temperature for 30 min. before being quenched with aqueous ammonium chloride solution (ice bath). Acidic workup gave the crude alcohol which was purified by flash chromatography (ether/light petroleum, 20:80) as a colourless viscous oil (48.8 mg;

85%) and as a 85:15 mixture of epimers. ν_{max} 3611 m, 3466 br m (OH), 3011 m, 2964 s, 2924 vs, 2872 s, 1456 m, 1380 m, 1236 w, 982 m, 951 m, cm^{-1}. ^1H NMR spectrum (200 MHz, $CDCl_3$) d 0.846 (3H, d, $J_{1',2'}$ = 6.5 Hz, H1'), 0.868 (3H, d, $J_{4\text{-}Me,4}$ = 6.3 Hz, 4-CH_3), 0.955 (3H, t, $J_{5',4'}$ = 7.4 Hz, H5'), 1.632 (3H, m, 7-CH_3), 2.479 (1H, br s, $W_{h/2}$ = 11.3 Hz, OH), 3.736 (1H, m, H3'), 5.188 (1H, m, H8). Mass spectrum: m/z 250 (M, 2%), 232 (M-H_2O, 22), 189 (37), 162 (100), 81 (41).

Example 3

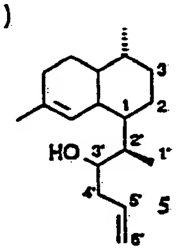
c) Phenyl alcohol (4)



The aldehyde (48 mg; 0.22 mmol) in ether (4 ml) was treated with phenyl magnesium bromide in ether as described above. Flash chromatography (ether/light petroleum, 20:80) of the crude product gave the phenyl alcohol (4) as a colourless viscous oil (55.8 mg; 86%). This was found to be a 76:24 mixture of epimers with respect to the hydroxyl group. Partial ^1H NMR spectrum (200 MHz, $CDCl_3$, *denotes minor epimer) δ *2.340 (1H, br s, $W_{h/2}$ = 11.3 Hz, OH), 2.512 (1H, br s, $W_{h/2}$=11.3 Hz, OH), *5.043 (1H, d, $J_{1',2'}$ = 4.3 Hz, H1'), 5.107 (1H, m, H1'), 5.171 (1H, m, H8), *5.277 (1H, m, H8).

Example 4

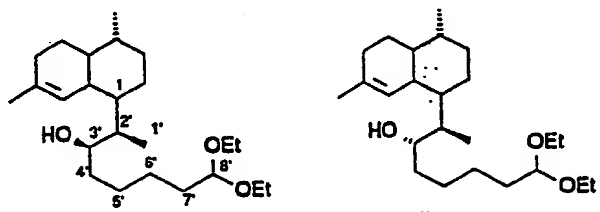
d) Allyl alcohol (5)



The aldehyde (95.1 mg; 0.43 mmol) in ether (5 ml) was treated with allylmagnesium bromide in ether as described above. Flash chromatography (ether/light petroleum, 20:80) of the crude product gave the allyl alcohol (5) as a colourless viscous oil (99.7 mg; 88%) and as an approximately 55:45 mixture of epimers. ^{1}H NMR spectrum (200 MHz, $CDCl_3$, *denotes minor epimer) δ 0.865 (3H, d, $J_{4\text{-Me},4} = J_{1',2'} = 6.2$ Hz, 4-CH_3, H1'), *0.881 (3H, d, $J_{4\text{-Me},4} = 6.5$ Hz, 4-CH_3), *0.897 (3H, d, $J_{1',2'} = 6.7$ Hz, H1'), 1.62 (3H, m, 7-CH_3, *7-CH_3), 2.48 (1H, br s, $W_{h/2}$ = 12 Hz, OH, *OH), 3.83-3.95 (1H, m, H3', *H3'), 5.07-5.29 (3H, m, 2 x H6', 2 x *H6', H8, *H8), 5.73-5.97 (1H, m, H5', *H5').

Example 5

e) Hydroxy acetals (6a) and (6b)



6a 6b

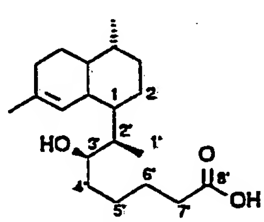
The aldehyde (210.7 mg; 0.96 mmol) in ether (8 ml) was treated with the Grignard reagent (1.5 equiv.), derived from 5-bromopentanaldehyde diethyl acetal and magnesium in THF, with cooling in an ice bath. The whole was stirred at room temperature for 30 min. before being quenched with aqueous ammonium chloride. The product was extracted into ether, and the ether extracts were washed with brine and dried (Na_2SO_4). Evaporation of the solvents under reduced pressure left a pale liquid which upon purification by flash chromatography (ether/light petroleum, 35:65) gave the hydroxy diethyl acetal as a colourless viscous oil (309 mg; 85%) and as a ca. 88:12 mixture of the (2'R,3'R)- and (2'R,3'S)-diastereomers, (6a) and (6b) respectively. These were separated by h.p.l.c. (ethyl acetate/light petroleum, 13:87, Whatman

Partisil 10, preparative) to give the (2'R,3'S)-isomer (6a) as the first to be eluted:- $[\alpha]_D^{20}$ +2.1° (c, 0.43, $CHCl_3$). ν_{max} ($CHCl_3$) 3536 w, 3600-3100 br s (OH), 2977 s, 2931 s, 2870 s, 1454 m, 1377 m, 1236 w, 1126 s, 1057 s, 993 m cm^{-1}. ^1H NMR spectrum (200 MHz, $CDCl_3$) δ 0.845 (3H, d, $J_{1',2'}$ = 6.4 Hz, H1'), 0.866 (3H, d, $J_{4\text{-Me},4}$ = 6.3 Hz, 4-CH_3), 1.205 (6H, t, J = 7.1 Hz, CH_3CH_2O), 1.631 (3H, m, 7-CH_3), 2.471 (1H, br s, $W_{h/2}$ = 11.5 Hz, OH), 3.41-3.72 (4H, m, CH_3CH_2O), 3.82 (1H, m, H3'), 4.491 (1H, t, $J_{8',7'}$ = 5.6 Hz, H8'), 5.180 (1H, m, H8). Mass spectrum: m/z 379 (M-1, 0.5%), 335 (10), 317 (11), 289 (16), 189 (47), 162 (100), 143 (43), 103 (66), 85 (52), 81 (61), 69 (53), 55 (66), 47 (57), 31 (62).

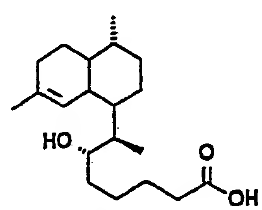
The next to be eluted was the (2'R,3'S)-isomer (6b):- $[\alpha]_D^{20}$ -7.3° (c, 0.30, $CHCl_3$). ν_{max} ($CHCl_3$) 3535 w, 3600 - 3100 br s (OH), 2977 s, 2934 s, 2873 s, 1455 m, 1377 m, 1234 w, 1126 s, 1057 s, 1002 m cm^{-1}. ^1H NMR spectrum (200 MHz, $CDCl_3$) δ 0.862 (6H, d, $J_{1',2'}$ = $J_{4\text{-Me},4}$ = 6.6 Hz, H1', 4-CH_3), 1.207 (6H, t, J = 7.1 Hz, CH_3CH_2O), 1.625 (3H, m, 7-CH_3), 2.461 (1H, br s, $W_{h/2}$ = 11.2 Hz, OH), 3.42-3.73 (4H, m, CH_3CH_2O), 3.82 (1H, m, H3'), 4.492 (1H, t, $J_{8',7'}$ = 5.6 Hz, H8'), 5.244 (1H, m, H8). Mass spectrum: m/z 379 (M-1, <0.1%), 335 (1), 317 (2), 189 (17), 162 (100), 143 (15), 103 (52), 98 (27), 95 (19), 85 (23), 81 (37), 75 (21), 69 (21), 55 (32), 43 (25), 31 (37).

Example 6

f) Hydroxy acid (7a) and (7b)



7a



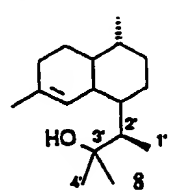
7b

The (2'R,3'R)-hydroxy acetal (6a) (40.4 mg; 0.11 mmol) was dissolved in THF (2 ml) and treated with aqueous HCl

(1 M, 1 ml) with rapid stirring at room temperature. After 30 min. the whole was extracted with ether and the combined extracts were washed with brine and dried (Na_2SO_4). Evaporation of the solvents left the crude aldehyde which was then dissolved in ethanol (1 ml). This was added to a stirred solution of silver nitrate (54 mg; 0.32 mmol) in water (0.12 ml). The resulting mixture was cooled in an ice bath, treated with aqueous KOH solution (34%, 0.105 ml), and then stirred at room temperature for 2 h. The black silver precipitate was filtered off, washing with water, and the filtrate was acidified with 3 M HCl with cooling in ice. The acidic solution was extracted with ether and the combined extracts were dried ($MgSO_4$) and evaporated to dryness. Purification of the residue by chromatography on silicic acid (ether/light petroleum, 1:1) afforded the (2'R,3'R)-hydroxy acid (7a) as a colourless viscous oil (31.2 mg; 91% overall). ^1H NMR spectrum (200 MHz, $CDCl_3$) δ 0.864 (3H, d, $J_{4\text{-Me},4}$ = 6.3 Hz, 4-CH_3), 0.881 (3H, d, $J_{1',2'}$ = 6.3 Hz, H1'), 1.648 (3H, m, 7-CH_3), 2.390 (2H, t, $J_{7',6'}$ = 7.3 Hz, H7'), 2.480 (1H, br s, $W_{h/2}$ = 12.8 Hz, OH), 3.86 (1H, m, H3'), 5.185 (1H, m, H8), 5.969 (1H, br s, $W_{h/2}$=88.5 Hz, COOH). Similarly, the (2'R,3'S)-hydroxy acetal (6b) (30.4 mg; 80 μmol) was converted into the (2'R,3'S)-hydroxy acid (7b) (24 mg; 93%). ^1H NMR spectrum (200 MHz, $CDCl_3$) δ 0.866 (3H, d, $J_{1',2'}$ = $J_{4\text{-Me},4}$ = 6.8 Hz, H1', 4-CH_3), 1.630 (3H, m, 7-CH_3), 2.382 (2H, t, $J_{7',6'}$ = 7.3 Hz, H7'), 2.461 (1H, br s, $W_{h/2}$ = 11.9 Hz, OH), 3.85 (1H, m, H3'), 4.435 (1H, br s, $W_{h/2}$ = 113 Hz, COOH), 5.238 (1H, m, H8).

<u>Example 7</u>

g) Dimethyl alcohol (8)



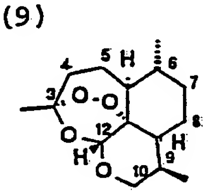
Methylmagnesium iodide was prepared by treating magnesium turnings (80 mg; 3.3 mmol) in dry ether (4 ml) with methyl iodide (0.17 ml; 2.8 mmol) under gentle reflux. A solution of the methyl ester of dihydroqinghao acid (138.3 mg; 0.55 mmol) in ether (3 ml) was then added dropwise at room temperature. The reaction mixture was heated under gentle reflux for a further 2 h before being cooled in ice. Aqueous ammonium chloride solution was added and the whole was extracted with ether. The combined ether extracts were washed with brine, dried (Na_2SO_4) and evaporated to dryness to give the crude alcohol. This was purified by flash chromatography (ether/light petroleum, 15:85) to give the dimethyl alcohol as a crystalline solid (113 mg; 82%), m.p. 52-54°. ^1H NMR spectrum (200 MHz, $CDCl_3$) δ 0.855 (3H, d, $J_{4-Me,4}$ = 6.4 Hz, 4-CH_3), 0.926 (3H, d, $J_{1',2'}$ = 6.9 Hz, H1'), 1.190 (3H, s, H4' or 3-CH_3), 1.249 (3H, s, H4' or 3-CH_3), 0.87 - 2.0 (13H, m), 1.628 (3H, br m, $W_{h/2}$ = 5.5 Hz, 7-CH_3), 2.526 (1H, br s, $W_{h/2}$ = 11.6 Hz, OH), 5.280 (1H, br m, $W_{h/2}$ = 6.8 Hz, H8). Mass spectrum: m/z 232 (M - H_2O, 9%), 217 (7), 189 (25), 163 (36), 162 (100), 147 (17), 121 (13), 107 (17), 95 (14), 81 (27), 59 (40), 41 (20).

PREPARATION OF FINAL COMPOUNDS

Preparation of Deoxoqinghaosu Derivatives

Example 8

a) Deoxoqinghaosu (9)



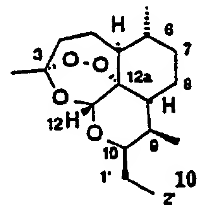
9

Dihydroqinghao alcohol (1) (43.9 mg; 0.20 mmol) was mixed with acetonitrile (2.5 ml) and irradiated under oxygen at -30° for 3 h in the presence of Rose Bengal sensitiser. The resulting hydroperoxide solution was then diluted

with dichloromethane (5 ml) and treated with $Cu(OTf)_2$ (0.020 mmol, 0.1 M in acetonitrile) at -20°. The reaction mixture was stirred at -15° for a further 40 min. and was then allowed to warm to room temperature over 20 min. before being cooled to -10°. Water was added and then the whole was extracted with ether. The combined extracts were washed with brine, dried ($MgSO_4$) and evaporated to dryness. The residue was purified by flash chromatography (ether/light petroleum, 40:60) to give deoxoqinghaosu (9) as a crystalline solid (19 mg; 36%). ^1H NMR spectrum (200 MHz, $CDCl_3$) δ 0.780 (3H, d, $J_{9\text{-}Me,9}$=7.2 Hz, 9-CH_3), 0.963 (3H, d, $J_{6\text{-}Me,6}$=5.9 Hz, 6-CH_3), 1.43 (3H, s, 3-CH_3), 3.449 (1H, dd, J_{gem}=11.7, $J_{10,9}$=11.7 Hz, H10), 3.731 (1H, br dd, J_{gem}=11.7, $J_{10,9}$=4 Hz, H10), 5.200 (1H, s, H12).

Example 9

b) Ethyl Deoxoqinghaosu (10)

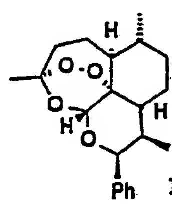


The ethyl alcohol (3) (77.9 mg; 0.31 mmol) was dissolved in dichloromethane (1 ml) and acetonitrile (2 ml) and irradiated under oxygen at -30° for 4 h in the presence of Rose Bengal sensitiser. The resulting solution of hydroperoxides was then diluted with dichloromethane (7 ml) and treated with $Cu(OTf)_2$ (0.031 mmol, 0.1 M in acetonitrile) at -20°. The mixture was kept at -20° for a further 40 min. and then allowed to warm to room temperature over 20 min. before being cooled to -10°. Water was added and then the whole was extracted into ether. The combined extracts were washed with brine, dried ($MgSO_4$) and evaporated to dryness. The residue was fractionated by flash chromatography (ether/light

petroleum, 10:90) to give ethyl deoxoqinghaosu (10) as a waxy solid (31.1 mg; 34%) and essentially only as the ß-epimer with respect to the ethyl group, ^1H NMR spectrum (400 MHz, $CDCl_3$) δ 0.860 (3H, d, $J_{Me,9}$=7.6 Hz, 9-CH_3), 0.958 (3H, d, $J_{Me,6}$=6.0 Hz, 6-CH_3), 1.034 (3H, t, $J_{2',1'}$=7.3 Hz, H2'), 1.426 (3H, s, 3-CH_3), 2.687 (1H, ddq, $J_{9,Me}$=7.5, $J_{9,8a}$=7.5, $J_{9,10}$=6.1 Hz, H9), 4.036 (1H, ddd, $J_{10,1'}$=10.6, $J_{10,9}$=6.0, $J_{10,1'}$=2.8 Hz, H10), 5.28 (1H, s, H12). ^{13}C NMR spectrum (50 MHz, $CDCl_3$) δ 12.105 (C2'), 13.01 (9-CH_3), 20.20 (6-CH_3), 22.27 (C8), 24.87 (C1' or C5), 24.87 (C1' or C5), 26.13 (3-CH_3), 30.25 (C9), 34.48 (C7), 36.57 (C4), 37.42 (C6), 44.45 (C8a), 52.03 (C5a), 77.64 (C10), 81.14 (C12a), 88.88 (C12), 103.14 (C3). Mass spectrum: m/z 296 (M^+, 0.7%), 278 (M-H_2O, 1), 264 (M-O_2, 24), 206 (100), 193 (33), 182 (45), 165 (35), 124 (72), 95 (35), 81 (34), 69 (58), 55 (92), 43 (90), 41 (63), 29 (38).

Example 10

c) Phenyl Deoxoqinghaosu (11)



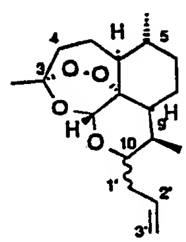
11

The phenyl alcohol (4) (55.4 mg; 0.19 mmol) was dissolved in dichloromethane (0.5 ml) and acetonitrile (1.5 ml) and photooxygenated at -30° for 3 h. Dilution of the reaction mixture with dichloromethane and treatment with $Cu(OTf)_2$ as described above followed by purification of the crude product by flash chromatography (ether/light petroleum, 7:93) afforded phenyl deoxoqinghaosu (11) as a viscous oil (17.4 mg; 27%) and exclusively as the ß-epimer with respect to the phenyl group, ^1H NMR spectrum (200 MHz, $CDCl_3$) δ 0.508 (3H, d, $J_{Me,9}$=7.7 Hz, 9-CH_3), 0.987 (3H, d, $J_{Me,6}$=5.6 Hz, 6-CH_3), 1.383 (3H, s, 3-

CH_3), 2.749 (1H, ddq, $J_{9,Me}$=7.1, $J_{9,8a}$=7.1, $J_{9,10}$=6.7 Hz, H9), 5.577 (1H, s, H12), 5.723 (1H, d, $J_{10,9}$=6.7 Hz, H10, 7.15-7.34 (5H, m, C_6H_5). ^{13}C NMR spectrum (50 MHz, $CDCl_3$) d 13.75 (9-CH_3), 19.98 (6-CH_3), 24.84 (C5 or C8), 25.01 (C5 or C8), 25.81 (3-CH_3), 32.21 (C9), 34.29 (C7), 36.77 (C4), 37.60 (C6), 43.59 (C8a), 51.59 (C5a), 73.14 (C10), 81.26 (C12a), 90.95 (C12), 102.38 (C3), 126.23, 126.38, 127.80 (C_6H_5 C_{meta}, C_{para}, C_{ortho}), 141.15 (C_6H_5 C_{ipso}). Mass spectrum: m/z 326 (M^+-H_2O, 0.4%), 312 (M-O_2, 13), 298 (12), 254 (16), 240 (23), 182 (100), 124 (68), 118 (52), 105 (31), 91 (32), 55 (23), 43 (46), 28 (61).

Example 11

d) Allyl Deoxoqinghaosu (12)



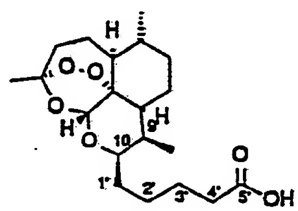
12

The allyl alcohol (5) (46 mg; 0.18 mmol) was submitted to the same conditions as described in a). Allyl deoxoqinghaosu (12) was then obtained by flash chromatography (ether/light petroleum, 10:90) as a crystalline solid (18.3 mg; 36%) and as a 1:3 mixture of α- and ß-epimers with respect to the allyl group, ^1H NMR spectrum (200 MHz, $CDCl_3$) δ 0.782 [3H, d, $J_{Me,9}$=7.2 Hz, 9-CH_3(α)], 0.888 [3H, d, $J_{Me,9}$=7.6 Hz, 9-CH_3(ß)], 0.951 [3H, d, $J_{Me,6}$=6.0 Hz, 6-CH_3(α)], 0.965 [3H, d, $J_{Me,6}$=5.8 Hz, 6-CH_3(ß)], 1.417 (3H, s, 3-CH_3), 2.69 [1H,ddq, $J_{9,8a}$=7.9, $J_{9,Me}$=7.3, $J_{9,10}$=6.3 Hz, H9(ß)], 3.491 [1H, ddd, $J_{10,1'}$=10.2, $J_{10,9}$=6.0, $J_{10,1'}$=3.7 Hz, H10(α)], 4.304 [1H, ddd, $J_{10,1'}$=10.0, $J_{10,9}$=6.1, $J_{10,1'}$=4.3 Hz, H10(ß)], 5.02-5.18 (2H, m, H3'), 5.249 [1H, s, H12(α)], 5.332 [1H, s, H12(ß)], 5.73-6.17 (1H, m, H2'). ^{13}C NMR spectrum (50 MHz, $CDCl_3$) δ

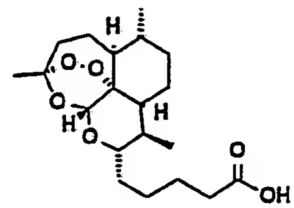
12.99 [9-CH_3(ß)], 13.60 [9-CH_3(α)], 20.20 [6-CH_3(ß)], 20.33 [6-CH_3(α)], 21.51 (CH_2), 24.72 (CH_2), 24.90 (CH_2), 26.09 (3-CH_3), 30.21 [C9(ß)], 31.03 [C9(α)], 34.23 [C7(ß)], 34.49 [C7(α)], 36.35 [C4(α)], 36.61 [C4(ß)], 37.16 (CH_2), 37.40 [C6(α)], 37.49 [C6(ß)], 44.33 [C8a(ß)], 46.07 [C8a(α)], 52.00 [C5a(α)], 52.36 [C5a(ß)], 73.87 [C10(α)], 74.73 [C10(ß)], 80.70 [C12a(α)], 81.08 [C12a(ß)], 89.10 [C12(ß)], 92.02 [C12(α)], 103.14 [C3(ß)], 103.97 [C3(α)], 116.08 [C3'(ß)], 116.42 [C3'(α)], 134.92 [C2'(α)], 136.48 [C2'(ß)].

Example 12

e) Carboxybutyl Deoxoqinghaosu (13a), (13b)



13a



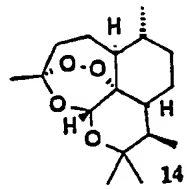
13b

The hydroxy acid (7a) (30.2mg; 93.7 μmol) was submitted to the same conditions as described in a). The resulting crude product was then fractionated by chromatography on silicic acid (ether/light petroleum, 50:50) to give the ß-epimer of carboxybutyl deoxoqinghaosu (13a) as a colourless viscous oil (11.9 mg; 34%). ^1H NMR spectrum (200 MHz, $CDCl_3$) δ 0.857 (3H, d, $J_{9\text{-Me},9}$=7.6 Hz, 9-CH_3), 0.961 (3H, d, $J_{6\text{-Me},6}$=5.8 Hz, 6-CH_3), 1.419 (3H, s, 3-CH_3), 4.150 (1H, ddd, $J_{10,1'}$=10.1, $J_{10,9}$=6.2, $J_{10,1'}$=2.2 Hz, H10), 5.298 (1H, s, H12). ^{13}C NMR spectrum (50 MHz, $CDCl_3$) δ 13.01 (9-CH_3), 20.19 (6-CH_3), 24.63, 24.63, 24.86 (C1', C5, C8), 26.10 (3-CH_3), 27.03, 29.01 (C3', C2'), 30.26 (C9), 33.88 (C4'), 34.45 (C7), 36.57 (C4), 37.44 (C6), 44.39 (C8a), 52.36 (C5a), 75.37 (C10), 81.13 (C12a), 88.99 (C12), 103.22 (C3), 178.98 (C5'). Similarly, the hydroxy acid (7b) (18.3 mg; 57 μmol) was converted into the α-epimer of carboxybutyl deoxoqinghaosu (13b) (8.4

mg; 40%). [1]H NMR spectrum (200 MHz, $CDCl_3$) d 0.777 (3H, d, $J_{9\text{-}Me,9}$=7.4 Hz, 9-CH_3), 0.953 (3H, d, $J_{6\text{-}Me,6}$=6.2 Hz, 6-CH_3), 1.409 (3H, s, 3-CH_3), 3.42 (1H, m, H10), 5.219 (1H, s, H12).

Example 13

f) Dimethyl Deoxoqinghaosu (14)



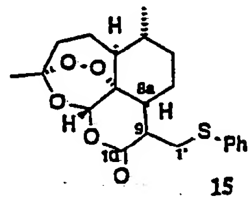
14

The dimethyl alcohol (8) (53 mg; 0.21 mmol) was submitted to the same conditions as described in a). Dimethyl deoxoqinghaosu (14) was then isolated by flash chromatography on silica (ether/light petroleum, 20:80) as a viscous oil which slowly crystallised (22 mg; 35%). ^{1}H NMR spectrum (200 MHz, $CDCl_3$) δ 0.887 (3H, d, $J_{9\text{-}Me,9}$ = 7.5 Hz, 9-CH_3), 0.959 (3H, d, $J_{6\text{-}Me,6}$ = 5.8 Hz, 6-CH_3), 1.228 (3H, s, 10-CH_3), 1.325 (3H, s, 10-CH_3), 1.424 (3H, s, 3-CH_3), 1.36-1.93 (9H, m), 2.027 (1H, ddd, J = 14.4, J = 4.7, J = 3.1 Hz), 2.277 (1H, ddd, J = 13.9, J = 13.4, J = 4.0 Hz), 2.433 (1H, dq, $J_{9,9\text{-}Me}$ = 7.5, $J_{9,8a}$ = 4.3 Hz, H9), 5.313 (1H, s, H12). ^{13}C NMR spectrum (50 MHz, $CDCl_3$) δ 13.96 (9-CH_3), 20.41 (6-CH_3), 20.76 (10-CH_3), 23.81 (C8), 24.56 (C5), 26.32 (3-CH_3), 31.91 (C9), 34.76 (C7), 35.68 (10-CH_3), 36.58 (C4), 37.48 (C6), 46.28 (C8a), 52.80 (C5a), 76.3 - 77.6 (C10, obscured by $CDCl_3$), 80.97 (C12a), 88.94 (C12), 103.84 (C3). Mass spectrum: m/z 264 (M-O_2, 7%), 250 (28), 235 (17), 207 (17), 206 (17), 192 (73), 177 (100), 165 (32), 138 (48), 123 (24), 109 (24), 95 (28), 81 (23), 69 (37), 55 (52), 43 (95), 28 (21).

Preparation of Dehydroqinghaosu Adducts

Example 14

a) With thiophenol

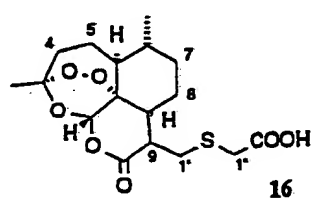


15

A solution of dehydroqinghaosu (17.6 mg; 62.7 μmol) in chloroform (2 ml) was treated with thiophenol (6.5 μl; 62.7 μmol) and triethylamine (4 μl) with cooling in an ice bath. The whole was then stirred at room temperature under nitrogen for 24 h. Aqueous workup afforded the phenylthio derivative (15) as a 1:2 mixture of ß- and α-epimers in essentially quantitative yield and free of by-products (23.9 mg; 98%). ^1H NMR spectrum (600 MHz, $CDCl_3$) δ 0.992 [3H, d, $J_{Me,6}$=6.3 Hz, 6-CH_3(α)], 1.007 [3H, d, $J_{Me,6}$=5.9 Hz, 6-CH_3(ß)], 1.419 [3H, s, 3-CH_3(ß)], 1.451 [3H, s, 3-CH_3(α)], 2.272 [1H, ddd, $J_{9,1'}$=9.4, $J_{9,1'}$=3.4, $J_{9,8a}$=1 Hz, H9(α)], 2.834 [1H, dd, J_{gem}=13.7, $J_{1',9}$=11.8 Hz, H1'(ß)], 3.139 [1H, dd, J_{gem}=13.8, $J_{1',9}$=11.7 Hz, H1'(α)], 3.446 [1H, ddd, $J_{9,1'}$=11.8, $J_{9,1'}$=5, $J_{9,8a}$=5 Hz, H9(ß)], 3.776 [1H, dd, J_{gem}=13.8, $J_{1',9}$=4.4 Hz, H1'(ß)], 3.860 [1H, dd, J_{gem}=13.8, $J_{1',9}$=3.4 Hz, H1'(α)], 5.864 [1H, s, H12(ß)], 5.918 [1H, s, H12(α)], 7.18-7.40 (5H, m, SPh).

Example 15

b) With thioglycolic acid.



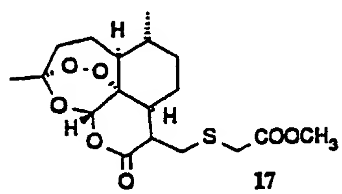
16

A solution of dehydroqinghaosu (14.4 mg; 51.4 μmol) in chloroform (2 ml) was treated with thioglycolic acid (3.6 μl; 51.4 μmol) and triethylamine (7.2 μl; 51.4 μmol).

The whole was stirred at room temperature under nitrogen for 3 d before being quenched with $NaHCO_3$ solution (0.3 M). The resulting mixture was extracted with ether and then the aqueous phase was acidified (HCl, 0.1M) to pH 6. Extraction of the aqueous phase with ether followed by the usual workup afforded the adduct (16) as a 2:1 mixture of ß- and α-epimers (15.4 mg; 81%). These were separated by chromatography on silicic acid (ether/light petroleum, 60:40) as very hygroscopic fine white solids. First was obtained the ß-epimer. ^{1}H NMR spectrum (200 MHz, $CDCl_3$) δ 1.007 (3H, d, $J_{6\text{-Me},6}$ = 5.6 Hz, 6-CH_3), 1.447 (3H, s, 3-CH_3), 5.878 (1H, s, H12). Next was obtained the α-epimer. ^{1}H NMR spectrum (200 MHz, $CDCl_3$) δ 1.004 (3H, d, $J_{6\text{-Me},6}$ = 5.6 Hz, 6-CH_3), 1.449 (3H, s, 3-CH_3), 5.956 (1H, s, H12).

Example 16

c) With methyl thioglycolate.



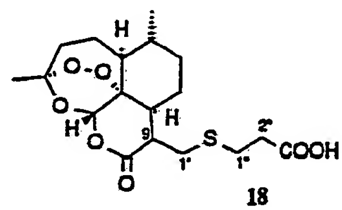
17

Dehydroqinghaosu (16.7 mg; 59.5 μmol) was treated with methyl thioglycolate (5.3 μl; 59.5 μmol) and triethylamine (4 μl) in chloroform (2 ml) for 3 d as described above. The reaction mixture was evaporated to dryness and then fractionated by flash chromatography (ether/light petroleum, 50:50) to give the ß-epimer as the least polar fraction (13.8 mg; 60%) and as very fine needles, ^{1}H NMR spectrum (200 MHz, $CDCl_3$) δ 1.00 (3H, d, $J_{Me,6}$=5.9 Hz, 6-CH_3), 1.44 (3H, s, 3-CH_3), 2.65 (1H, dd, J_{gem}=13.2, $J_{1',9}$=10.6 Hz, H1'), 3.21 (1H, d, J_{gem}=14.9 Hz, H1"), 3.32 (1H, d, J_{gem}=14.9 Hz, H1"), 3.40 (1H, dd, J_{gem}=13.1, $J_{1',9}$=5.0 Hz, H1'), 3.51 (1H, ddd, $J_{9,1'}$=10.8, $J_{9,1'}$=5.0, $J_{9,8a}$=5 Hz, H9), 3.757 (3H, s, OCH_3), 5.871 (1H,

s, H12), and the α-epimer (6.4 mg; 28%) as a viscous oil, ^{1}H NMR spectrum (200 MHz, $CDCl_3$) δ 1.00 (3H, d, $J_{6\text{-Me},6}$ = 5.3 Hz, 6-CH_3), 1.45 (3H, s, 3-CH_3), 1.2 - 2.5 (11H, m), 2.39 (1H, ddd, $J_{9,1'}$ = 11.2, $J_{9,1'}$ = 4.0, $J_{9,8a}$ = 1.2 Hz, H9), 3.00 (1H, dd, J_{gem} = 13.3, $J_{1',9}$ = 11.3 Hz, H1'), 3.25 (1H, d, J_{gem} = 14.9 Hz, H1"), 3.32 (1H, d, J_{gem} = 14.9 Hz, H1"), 3.41 (1H, dd, J_{gem} = 13.2, $J_{1',9}$ = 3.9 Hz, H1'), 3.74 (3H, s, OCH_3), 5.95 (1H, s, H12).

Example 17

d) With mercaptopropionic acid



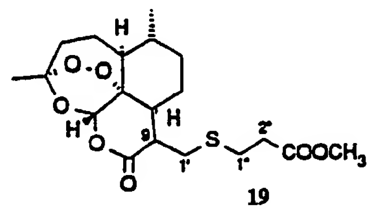
18

A solution of dehydroqinghaosu (24.9 mg; 89 μmol) in chloroform (2 ml) was treated with mercaptopropionic acid (7.8 μl; 89 μmol) and triethylamine (12.4 μl; 89 μmol) with stirring at room temperature under nitrogen for 24 h and then for a further 24 h allowing the solvent to slowly evaporate away. The whole was diluted with ether and washed with dilute HCl solution. The ether solution was dried ($MgSO_4$) and evaporated to dryness to give the crude adduct which was submitted to chromatography on silicic acid (ether/light petroleum, 75:25). The first compound to be eluted was the ß-epimer which was obtained as a fine white solid (8.2 mg; 24%). ^{1}H NMR spectrum (600 MHz, $CDCl_3$) δ 1.008 (3H, d, $J_{6\text{-Me},6}$ = 5.7 Hz, 6-CH_3), 1.041 - 1.155 (2H, m), 1.40 - 1.51 (4H, m), 1.445 (3H, s, 3-CH_3), 1.76 - 1.83 (2H, m), 2.141 (1H, ddd, J = 13.0, J = 4.6, J = 4.6 Hz), 2.42 - 2.47 (1H, m), 2.554 (1H, dd, J_{gem} = 13.5, $J_{1',9}$ = 11.9 Hz, H1'), 2.67 - 2.70 (2H, m, 2 X H2"), 2.79 - 2.81 (2H, m, 2 X H1"), 3.334 (1H, dd, J_{gem} = 13.6, $J_{1',9}$ = 4.6 Hz, H1'), 3.449 (1H, ddd, $J_{9,1'}$ = 11.6,

$J_{9,1'}$ = 4.8, $J_{9,8a}$ = 4.8 Hz, H9), 5.865 (1H, s, H12). Next was obtained the α-epimer as a viscous oil (24.5 mg; 71%). ^{1}H NMR spectrum (200 MHz, $CDCl_3$) δ 1.060 (3H, d, $J_{6\text{-Me},6}$ = 5.8 Hz, 6-CH_3), 1.506 (3H, s, 3-CH_3), 1.15 - 2.61 (11H, m, 2 X H4, 2 X H5, H5a, H6, 2 X H7, 2 X H8, H8a), 2.355 (1H, ddd, $J_{9,1'}$ = 11.5, $J_{9,1'}$ = 3.7, $J_{9,8a}$ = 1.0 Hz, H9), 2.70 - 2.90 (4H, m, 2 X H1", 2 X H2"), 2.952 (1H, dd, $J_{1',1'}$ = 13.3, $J_{1',9}$ = 11.6 Hz, H1'), 3.401 (1H, dd, $J_{1',1'}$ = 13.3, $J_{1',9}$ = 3.7 Hz, H1'), 5.96 (1H, s, H12).

Example 18

e) With methyl mercaptopropionate



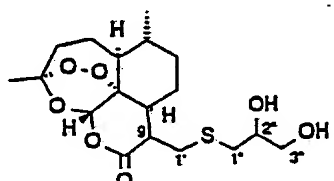
19

Dehydroqinghaosu (29.4 mg; 0.105 mmol) in chloroform (3 ml) was treated with methyl mercaptopropionate (11.6 μl; 0.105 mmol) and triethylamine (4 μl) with stirring as described above. After 48 h the whole was evaporated to dryness and the residue was submitted to flash chromatography (ether/light petroleum, 50:50) to give first the ß-epimer as a fine white solid (18.3 mg; 44%). ^{1}H NMR spectrum 200 MHz, $CDCl_3$) δ 1.01 (3H, $J_{6\text{-Me},6}$ = 5.8 Hz, 6-CH_3), 1.45 (3H, s, 3-CH_3), 1.0- 1.9 (6H, m), 2.0 - 2.2 (3H, m), 2.4 - 2.5 (2H, m), 2.54 (1H, dd, J_{gem} = 13.2, $J_{1',9}$ = 11.4 Hz, H1'), 2.59 - 2.67 (2H, m, 2 X H2"), 2.76 - 2.84 (2H, m, 2 X H1"), 3.33 (1H, dd, J_{gem} = 13.3, $J_{1',9}$ = 4.7 Hz, H1'), 3.44 (1H, ddd, $J_{9,1'}$ = 11.5, $J_{9,1'}$ = 4.9, $J_{9,8a}$ = 4.9 Hz, H9), 3.71 (3H, s, OCH_3), 5.67 (1H, s, H12). ^{13}C NMR spectrum (50 MHz, $CDCl_3$) δ 19.76 (6-CH_3), 22.97 (C7), 24.84 (C8), 25.12 (3-CH_3), 26.98 (CH_2), 28.86 (CH_2), 33.30 (CH_2), 34.41 (CH_2), 35.83 (CH_2), 37.51 (C6), 37.55 (C8a),

41.17 (C5a), 49.97 (C9), 51.83 (OCH_3), 79.24 (C12a), 93.80 (C12), 105.49 (C3), 170.17 ($COOCH_3$), 172.08 (C10). Next was obtained the α-epimer as a viscous oil (9.7 mg; 23%). ^1H NMR spectrum (200 MHz, $CDCl_3$) δ 1.00 (3H, d, $J_{6\text{-}Me,6}$ = 5.7 Hz, 6-CH_3), 1.1 - 1.6 (5H, m), 1.45 (3H, s, 3-CH_3), 1.65 -1.83 (2H, m), 1.69 -2.08 (2H, m), 2.14 (1H, br dd, J_{gem} = 13.7, J = 4.5 Hz), 2.29 (1H, ddd, $J_{9,1'}$ = 11.6, $J_{9,1'}$ = 3.7, $J_{9,8a}$ = 1.1 Hz, H9), 2.3 - 2.47 (1H, m), 2.59 - 2.67 (2H, m, 2 X H2"), 2.76 - 2.84 (2H, m, 2 X H1"), 2.88 (1H, dd, J_{gem} = 13.2, $J_{1',9}$ = 11.6 Hz, H1'), 3.33 (1H, dd, J_{gem} = 13.2, $J_{1',9}$ = 3.7 Hz, H1'), 3.70 (3H, s, OCH_3), 5.95 (1H, s, H12). When the solvent was changed to dichloromethane the ß-epimer was obtained in only 15% yield and the α-epimer in 77% yield.

Example 19

f) With thioglycerol



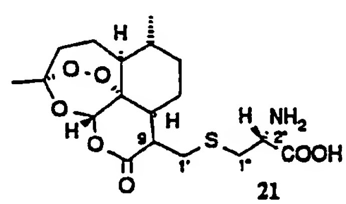
20

Dehydroqinghaosu (14.6 mg; 52 μmol) in chloroform (2 ml) was treated with thioglycerol (5.6 mg; 4.3 μl; 52 μmol) and triethylamine (4 μl). The whole was stirred at room temperature overnight after which time complete reaction had taken place. The reaction mixture was evaporated to dryness to give the crude ß- and α-epimeric adducts in a ratio of 2.2 : 1. These were separated by flash chromatography on silica (ethyl acetate/light petroleum, 80 : 20) to give first, the ß-epimer as a mixture of diastereomers at C2" and as a fine white solid (12.2 mg; 60%). ^1H NMR spectrum (400 MHz, $CDCl_3$) (*denotes other diastereomer) δ 1.012 (3H, d, $J_{6\text{-}Me,6}$ = 5.7 Hz, 6-CH_3), 1.07 - 1.14 (2H, m), 2.00 - 2.18 (3H, m), 2.40 - 2.48

(1H, m), 2.54 - 2.74 (3H, m), 3.320 (1H, ddd, J = 13.1, J = 7.9, J = 4.8 Hz), *3.473 (1H, ddd, $J_{9,1'}$ = 11.2, $J_{9,1'}$ = 5.9, $J_{9,8a}$ = 3.6 Hz, H9), 3.482 (1H, ddd, $J_{9,1'}$ = 10.9, $J_{9,1'}$ = 5.4, $J_{9,8a}$ = 5.4 Hz, H9), *3.572 (1H, dd, J = 11.2, J = 5.8 Hz), 3.580 (1H, dd, J = 11.2, J = 5.9 Hz), 3.758 (1H, br ddd, J = 11.3, J = 3.0, J = 3.0 Hz), 3.82 -3.88 (1H, m), 5.878 (1H, s, H12). ^{13}C NMR spectrum (50 MHz, $CDCl_3$) δ 19.79 (6-CH_3), 23.17 (C8), 24.85 (C5), 25.14 (3-CH_3), 28.92, 29.72 (CH_2), 30.94 (C9), 33.35 (C7), 35.87 (C6), 37.53 (CH), 37.80, 38.19 (CH), 41.52, 41.73 (CH), 50.01 (C5a), 65.29 (C3"), 69.74, 70.37 (C2"), 79.29 (C12a), 93.89 (C12), 105.59 (C3), 170.25 (C10). The α-epimer was obtained as a viscous oil and as a mixture of diastereomers at C2" (5.1 mg; 25%). ^1H NMR spectrum (400 MHz, $CDCl_3$) δ 1.009 (3H, d, $J_{6\text{-Me},6}$ = 6.1 Hz, 6-CH_3), 1.16 - 1.34 (2H, m), 1.39 - 1.52 (2H, m), 1.452 (3H, s, 3-CH_3), 1.70 - 1.82 (2H, m), 1.92 - 2.16 (3H, m), 2.31 - 2.43 (2H, m), 2.59 - 2.75 (3H, m), 2.933 (1H, ddd, J = 14.0, J = 11.1, J = 3.1 Hz), 3.329 (1H, ddd, J = 13.2, J = 13.2, J = 4.0 Hz), 3.55 - 3.60 (1H, m), 3.766 (1H, br d, J = 11.4 Hz), 3.81 - 3.89 (1H, m), 5.954 (1H, s, H12).

Example 20

g) With L-cysteine



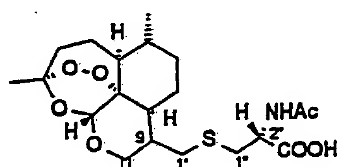
21

L-Cysteine (5.8 mg; 36.8 μmol) was added as a solid to a stirred solution of dehydroqinghaosu (10.3 mg; 36.8 μmol) in methanol (1.5 ml) under nitrogen. Complete conversion to the adduct occurred almost immediately. The reaction mixture was then evaporated to dryness whereupon the crude residue was triturated with ether and the resulting

fine white powder was obtained by filtration. The adduct (17), so obtained, was found to be only sparingly soluble in methanol and insoluble in most other solvents. Therefore, an estimate of the epimer ratio could not be made. ^1H NMR spectrum (200 MHz, CD_3OD) δ 0.87 [3H, d, $J_{Me,6}$=6.4 Hz, 6-CH_3(ß)], 1.006 [3H, d, $J_{6\text{-}Me}$=5.8 Hz, 6-CH_3(α)], 1.390 [3H, s, 3-CH_3(ß)], 1.396 [3H, s, 3-CH_3(α)], 2.821 [1H, dd, $J_{1',1'}$ = 12.9, $J_{1',9}$ = 10.0 Hz, H1'(ß)], 2.951 [1H, dd, $J_{2'',2''}$ = 14.6, $J_{2'',1''}$ = 8.5 Hz, H2"(α)], 2.966 [1H, dd, $J_{2'',2''}$ = 14.4, $J_{2'',1''}$ = 8.5 Hz, H2"(ß)], 3.167 [1H, dd, $J_{2'',2''}$ = 14.6, $J_{2'',1''}$ = 4.0 Hz, H2"(α)], 3.191 [1H, dd, $J_{2'',2''}$ = 14.4, $J_{2'',1''}$ = 4.0 Hz, H2"(ß)], 3.490 [1H, ddd, $J_{9,1'}$ = 10.0, $J_{9,1'}$ = 5.4, $J_{9,8}$ = 5.4 Hz, H9(ß)], 3.702 [1H, dd, $J_{1'',2''}$ = 8.6, $J_{1'',2''}$ = 4.0 Hz, H1"(ß)], 3.749 [1H, dd, $J_{1'',2''}$ = 8.4, $J_{1'',2''}$ = 3.7 Hz, H1"(α)], 6.026 [1H, s, H12(ß)], 6.127 [1H, s, H12(α)]

Example 21

h) With N-acetyl-L-cysteine



22

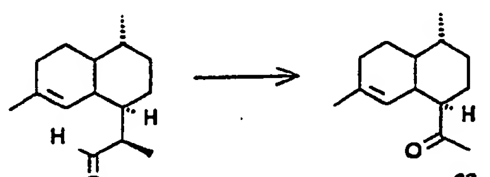
Triethylamine (7.9 μl; 57 μmol) was added to a stirred solution of dehydroqinghaosu (15.9 mg; 57 μmol) and a suspension of N-acetyl-L-cysteine (9.3 mg; 57 μmol) in chloroform (3 ml). Deprotonation of the cysteine caused it to go into solution. The reaction mixture was stirred under nitrogen for 24 h and then for a further 24 h allowing the solvent to evaporate away slowly. Water was added and then the whole was extracted once with ether. The aqueous layer was separated and then acidified with 1 M hydrochloric acid in the presence of ether and extracted three times with more ether. The combined

extracts were dried ($MgSO_4$) and evaporated to dryness to give the cysteine adducts as a 1:1 mixture of α- and ß-epimers and as a viscous oil (18.9 mg; 75%). ^1H NMR spectrum (200 MHz, $CDCl_3$) δ 1.00 (3H, d, $J_{6\text{-Me},6}$ = 5.4 Hz, 6-CH_3), 1.44 (3H, s, 3-CH_3), 2.10 (3H, s, acetyl), 4.82 (1H, br m, $W_{h/2}$ = 15.7 Hz, N-H), 5.875 [1H, s, H12(ß)], 5.961 [1H, s, H12(α)], 6.822 (1H, dd, $J_{2'',1''}$ = 7.9, $J_{2'',1''}$ = 7.9 Hz, H2"), 7.45 (1H, br s, $W_{h/2}$ = 28.6 Hz, COOH).

Preparation of Ring-Contracted Analogues of Qinghaosu Derivatives

Example 22

a) Oxidative degradation of dihydroqinghao aldehyde (2)

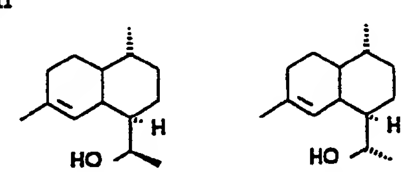


2 23

A mixture of dihydroqinghao aldehyde (2) (105 mg; 0.48 mmol), DABCO (26 mg), cupric acetate monohydrate (50 mg) and 2,2'-bipyridyl (50 mg) in DMF (7 ml) was stirred for 12 h at 70-75° and then the solvent was removed under vacuum. The resulting residue was submitted to flash chromatography (ether/light petroleum, 1:5) to afford the ketone (23) as a colourless viscous oil (77 mg; 78%) $[\alpha]_D^{18}$-44.4° (c, 2.03, $CHCl_3$). ^1H NMR spectrum (400 MHz, $CDCl_3$) δ 0.94 (3H, d, J=6 Hz), 1.65 (3H, s), 2.20 (3H,s), 2.95 (1H, br s), 4.48 (1H, s).

Example 23

b) Reduction

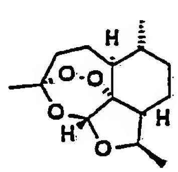


24a 24b

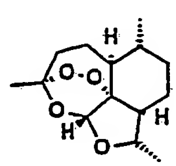
The ketone (23) (98 mg; 0.47 mmol) in methanol (3 ml) was treated with $NaBH_4$, portionwise, at 0^o until a t.l.c. monitor showed that the reaction was complete. Water was added and the whole was thoroughly extracted with ether. The combined ether extracts were washed with brine and dried (Na_2SO_4) and evaporated to dryness. The residue was then submitted to flash chromatography (ether/light petroleum, 2:8) to give the alcohols, (24a) and (24b) as a colourless viscous oil (98 mg; 99%) and as a 1:4 mixture of epimers. These were separated by h.p.l.c. (ethyl acetate/light petroleum, 8:92, Whatman partisil 10 M9, semi-preparative) to give, firstly, epimer (24b) $[\alpha]_D^{18}$-10.3^o (c, 2.34, $CHCl_3$). ^1H NMR spectum (400 MHz, $CDCl_3$) δ 0.88 (3H, d, J=6 Hz), 1.24 (3H, d, J=6 Hz), 1.63 (3H, br s), 2.72 (1H, br s), 3.81 (1H, dq), 5.34 (1H, s). The next to be eluted was epimer (24a) $[\alpha]_D^{18}$-8.5^o (c, 1.68, $CHCl_3$). ^1H NMR spectrum (400 MHz, $CDCl_3$) δ 0.89 (3H, d, J=6 Hz), 1.26 (3H, d, J=6.4 Hz), 1.62 (3H, br s), 2.46 (1H, br s), 3.775 (1H, dq), 5.14 (1H, s).

Example 24

c) Oxygenation to deoxoqinghaosu analogues (25a) and (25b)



25a



25b

The major alcohol epimer (24b) (55.8 mg; 0.27 mmol) in acetonitrile (2 ml) and dichloromethane (1 ml) was irradiated under oxygen at -30^o for 4 h in the presence of Rose Bengal sensitiser. The resulting hydroperoxide solution was then diluted with dichloromethane (7 ml) and treated with $Cu(OTf)_2$ (0.027 mmol, 0.1 M in acetonitrile) as described for the preparation of the

deoxoqinghaosu derivatives. The crude product mixture obtained after work-up was submitted to flash chromatography (ether/light petroleum, 1:6) to give the five membered ring analogue (25b) as a colourless viscous oil (19.1 mg; 28%) $[\alpha]_D^{20}$+120.2° (c, 1.1, $CHCl_3$). ^1H NMR spectrum (400 MHz, $CDCl_3$) δ 0.98 (3H, d, J=6 Hz), 1.45 (3H, s), 1.47 (3H, d, J=6.5 Hz), 3.93 (1H, dq), 5.59 (1H, s). Similarly, the minor alcohol epimer (24a) (20 mg; 96 μmol) was converted into the five membered analogue (25a) (7.8 mg; 32%). ^1H NMR spectrum (400 MHz, $CDCl_3$) δ 0.98 (3H, d, J=6 Hz), 1.24 (3H, d, J=6.4 Hz), 1.44 (3H, s), 4.92 (1H, dq), 5.67 (1H,s).

Activity data

The testing results for the qinghaosu derivatives are as follows:

Ethyl deoxoqinghaosu has been tested against two strains of *Plasmodium falciparum* concurrently with artemether and sodium artesunate, the latter two compounds being in clinical use at the present time. The results are shown in the following graphs.

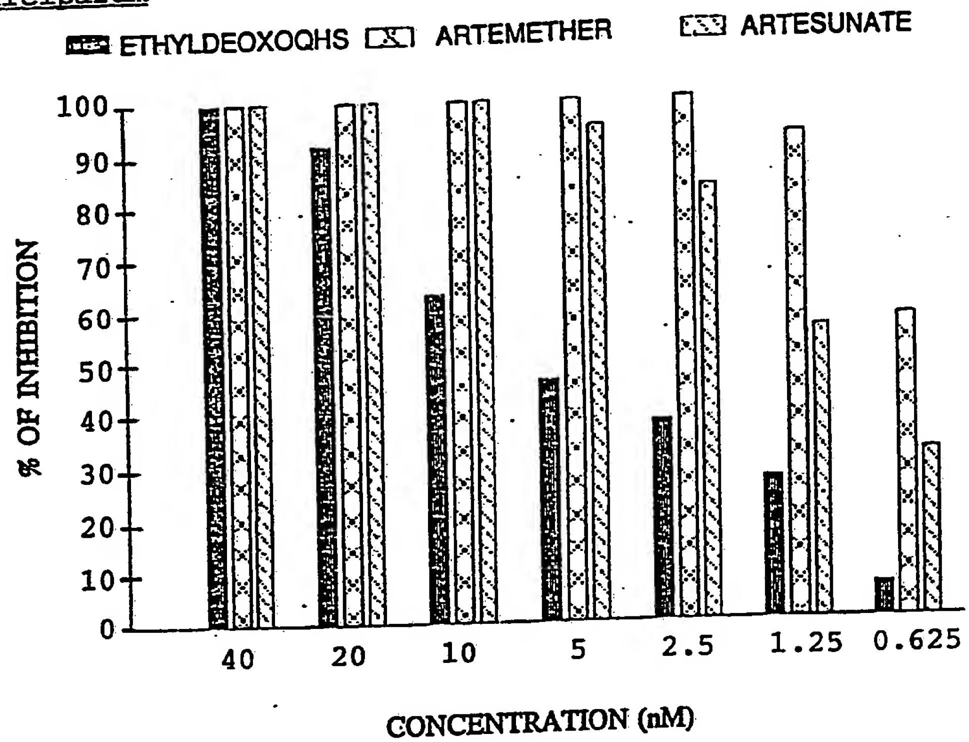
K1 strain from Kanchanaburi, Thailand (chloroquine resistant)

IC_{50} = 6.0 nM = 2.0 ng/ml

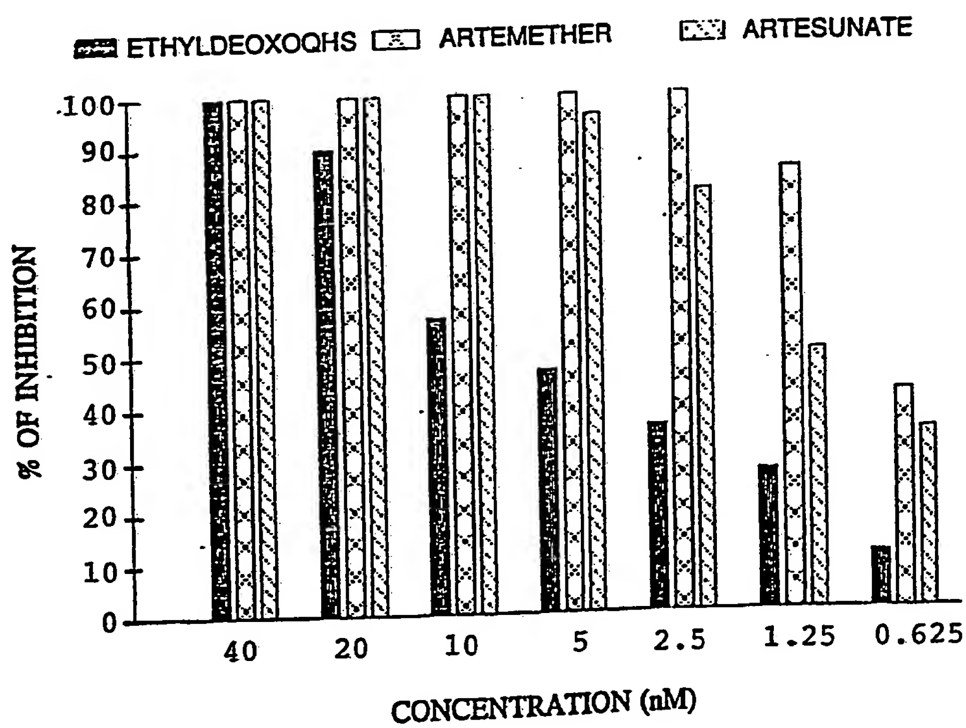
FC27 strain from Madang, Papua New Guinea (chloroquine sensitive)

IC_{50} = 4.3 nM = 1.3 ng/ml

In vitro effects of ethyldeoxoqinghaosu, artemether and artesunate against the FC27 strain of *Plasmodium falciparum*



In vitro effects of ethyldeoxoqinghaosu, artemether and artesunate against the K1 strain of *Plasmodium falciparum*



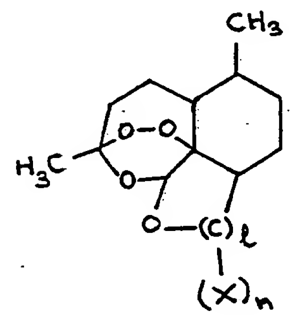
Effect of qinghaosu analogues on *Toxoplasma gondii in vitro**

QHS Analogue	IC50 (μM)
9 Deoxoqinghaosu	0.28
qinghaosu (natural compound)	0.9
qinghaosu (prepared from qinghao acid)	0.85
Dehydroqinghaosu sample 1	1.7
Dehydroqinghaosu sample 2	1.7
17 ß-epimer	2.2
19 ß-epimer	2.4
19 α-epimer	4.4
18 ß-epimer	4.8
18 α-epimer	14
Pyrimethamine (known compound)	1

* [3H]-uracil incorporation assay

Claims

1. Compounds of formula (I), pharmaceutically acceptable salts thereof or stereoisomeric forms thereof



(I)

wherein

l = 1, 2 or 3

n is an integer from 1-6

where X is independently selected from

H, =O, $=CH_2$, aryl, COR, OR, COOR or

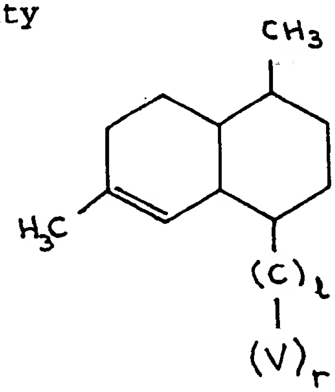
X = $-(CR_1R_2)_r-R_3$ where r is an integer from 1-10 and where r > 1, optionally at least 1 carbon atom can be replaced by O, S or N;

R_1, R_2 and R_3 are independently selected from

H; alkyl, alkenyl, alkynyl, aryl, each optionally substituted by one or more substituents selected from alkyl, alkenyl, alkynyl, aryl, halogen, OR, CF_3, NO_2, COOR, NRR', SR, COR, CONRR', SO_3R, SO_2NRR', SR, SOR, and SO_2R, where R and R' are independently selected from H, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted aryl or optionally substituted arylalkyl wherein the optional substituents are as defined above; provided that $(C)_l-(X)_n$ is not $-CH_2-CH(CH_3)-$.

2. A process for preparing compounds of formula (I), pharmaceutically acceptable salts thereof or stereoisomeric forms thereof comprising:

(A) carrying out at least one addition reaction on a compound of formula (II) having a -C=O functionality



(II)

to provide another compound of formula (II) with an alcoholic functionality at the $(C)_l$-$(V)_r$ group and then followed by oxygenation to give a compound of formula (I); where V is as defined for X; r and l are as defined above;

(B) directly oxygenating a compound of formula (II) where there is a carboxylic functionality at the $(C)_l$-$(V)_r$ reactive chain and also a further functional group to facilitate subsequent addition reaction, to provide compounds of formula (I) and then carrying out a suitable addition reaction to provide the required side chain; or

(C) for compounds of formula (I) where l=1; carrying out one or more of the following steps on a compound of general formula (II):

(i) oxidative degradation and

(ii) reduction

followed by oxygenation to give compounds of formula (I).

3. A process according to claim 2 wherein the oxygenation step in process A, B or C comprises

(i) initial irradiation under oxygen in the presence of a catalyst such as Rose Bengal and

(ii) further oxygenation by treatment with a transition metal catalyst.

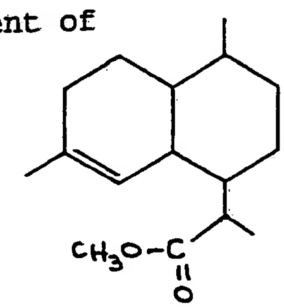
4. A process according to claim 3 wherein the transition metal catalyst is one or more selected from $Cu(OSO_2CF_3)_2$, copper(II) carboxylic salts and iron(III) salts.

5. A process according to claim 4 wherein the Cu(II) carboxylic salt is Cu(II) propionate or copper(II) 2-ethylhexanoate and the iron(III) salt is $Fe(phenanthroline)_3(PF_6)_3$.

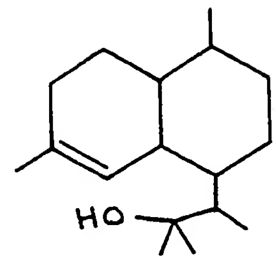
6. A process according to claim 5 wherein process step A comprises

(i) treatment of the aldehyde 2 with A-MgBr, where A is alkyl, aryl or alkenyl to provide the respective derivatives; followed by oxygenation to provide a compound of formula (I);

(ii) treatment of



with MeMgI to provide



followed by oxygenation to provide a compound of formula (I); or

(iii) treatment of the aldehyde 2 with $(EtO)_2CH$-A-MgBr where A is alkyl;
followed by hydrolysis and then oxidation to the HO(O=)C-A derivative;
followed by oxygenation to provide a compound of formula (I).

7. A process according to claim 5 wherein in process B the starting compound of formula (II) is dehydroqinghao acid which is oxygenated to provide dehydroqinghaosu and the addition reaction is carried out by treating dehydroqinghaosu with thiol nucleophiles.

8. A process according to claim 7 wherein the thiol nucleophile is thiophenol, thioglycolic acid, methyl thioglycolate, mercaptopropionic acid, methyl mercaptopropionate, thioglycerol, L-cysteine or N-acetyl-L-cysteine.

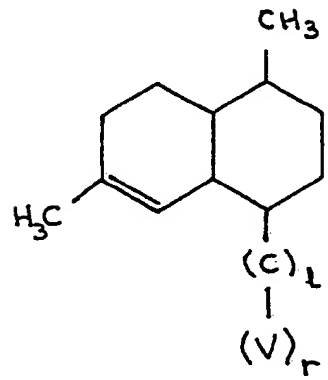
9. A process according to claim 5 wherein process (C) comprises

(i) oxidative degradation of aldehyde 2

(ii) reduction to the alcohol

(iii) followed by oxygenation to provide a compound of formula (I).

10. Compounds of general formula (II)



(II)

wherein V is as defined for X; r and l are as hereinbefore defined; provided that the $(C)_l-(V)_r$ group is not $CH(CH_3)CH_2OH$ and $CH(CH_3)C(=O)H$.

11. A pharmaceutical composition comprising a compound of formula (I), a pharmaceutically acceptable salt thereof or stereoisomeric forms thereof in a pharmaceutically acceptable carrier and/or diluent.

12. A method of treatment or prophylaxis of parasitic or viral diseases in a mammal comprising administering to the mammal a compound of formula (I), a pharmaceutically acceptable salt thereof or a stereoisomeric form thereof.

13. Use of a compound of formula (I), a pharmaceutically acceptable salt thereof or a stereoisomeric form thereof in the manufacture of a medicament for the treatment or prophylaxis of parasitic or viral diseases.

14. A compound of formula (I) substantially as herein described with reference to any one of examples 9-21 or 24.

A. CLASSIFICATION OF SUBJECT MATTER	
Int. Cl.^{5} C07D 493/20, C07C 33/38, 33/14, 57/26, 43/315, 59/46, 49/553	
According to International Patent Classification (IPC) or to both national classification and IPC	
B. FIELDS SEARCHED	
Minimum documentation searched (classification system followed by classification symbols) IPC: C07D 493/18, 493/20, C07C 33/38, 33/14, 57/26, 43/315, 59/46, 49/553	
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched AU: IPC as above	
Electronic data base consulted during the international search (name of data base, and where practicable, search terms used)	

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X	AU,B,42421/89 (629571) (HOECHST AG) 12 April 1990 (12.04.90) claim 1	1
P,X	AU,A,76185/91 (HOECHST AG) 7 November 1991 (07.11.91) claim 1	1
X	WO,A,8804660 (SRI INTERNATIONAL) 18 December 1986 (18.12.86) compound 13	1
A	Tetrahedron, vol. 45, no. 23, 1989, Ye Bin et al: "Synethesis of carba-analogues of Qinghaosu", pages 7287-7290	1-14

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search 22 January 1993 (22.01.93)	Date of mailing of the international search report 08 FEB 1993 (08.02.93)
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200 WODEN ACT 2606 AUSTRALIA Facsimile No. 06 2853929	Authorized officer R. DALBON Telephone No. (06) 2832372

C(Continuation).	DOCUMENTS CONSIDERED TO BE RELEVANT	
Category*	Citation of document, with indication, where appropriate of the relevant passages	Relevant to Claim No.
A	AU,A,64475/90 (UNIVERSITY OF SYDNEY) 18 April 1991 (18.04.91) whole document	1-14
A	AU,B,44675/89 (620937) (DERMATOLOGIC RESEARCH CORPORATION) 1 August 1991 (01.08.91) whole document	1-14

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report	Patent Family Member		
AU,B, 42421/89	CN,A, 1041595	EP,A1, 362730	JP,A2, 2145586
	NZ,A, 230868	ZA,A, 8907508	
AU,A, 76185/91	BR,A, 9101822	EP,A1, 456149	IL,A0, 98071
	JP,A2 4225983	PT,A, 97577	ZA,A, 9103393
WO,A, 8804660	CH,A, 679486	DE,T, 3790698	EP,A1, 346356
	GB,B2, 2218088	JP,T2, 1501710	SE,A, 8802906
	SE,A, 9000377	US,A, 4963683	US,A, 5019596
AU,A, 64475/90	BR,A, 9007687	EP,A1, 494209	FI,A, 921376
	HU,A0, 9200900	NO,A, 921178	WO,A1, 9104970
AU,B, 44675/89	US,A, 4978676		

END OF ANNEX